35. (once amended) The method of claim 33 further comprising a step of permeabilizing the host cell expressing the fusion polypeptide.

Please add new claim 36.

36. (newly added) The nucleic acid of claim 1, wherein the accessory enzyme is a pyrophosphorylase.

REMARKS

Claims 1-35 are pending in the present application. In this response, claims 1, 9, 23, 26, 27, and 33-35 are amended to more clearly define the claimed invention; claim 4 is canceled without prejudice to subsequent revival. Claims 28-32 are canceled without prejudice because, in the parent application, the Examiner withdrew the claims from consideration as allegedly being drawn to a non-elected invention. Claim 36 is added as a new claim. For convenience, the Examiner's rejections are addressed in the order presented in the November 20, 2001 Office Action. Appendix A provides the version with markings to show changes made. Also for the Examiner's convenience, Appendix B, listing all pending and amended claims, is included.

Specifically, claim 1 is amended to recite an "isolated" nucleic acid encoding a fusion protein comprising a) a catalytic domain of a glycosyltransferase "that catalyzes the transfer of a saccharide, from a saccharide donor comprising a nucleotide sugar, to an acceptor molecule" and b) a catalytic domain of an accessory enzyme "that catalyzes the formation of the nucleotide sugar." Support for this amendment is provided throughout the specification, for example, at page 3, line 21, to page 5, line 15. Claim 9 is amended to correct for minor editorial error and to more clearly define the recited group of accessory enzymes. Claim 23 is amended to more clearly define that the recited catalytic domains are "joined" by a peptide linker. Support for this amendment is provided, for example, at page 26, line 26, to page 27 line 8 of the specification. Claim

26 is amended to correct for minor editorial error, replacing "a" nucleic acid of claim 1 with "the" nucleic acid of claim 1. Claims 27 and 33 are amended to depend from claim 26 which is directed to an expression vector comprising the nucleic acid of claim 1. Support for this amendment is provided throughout the specification, for example, at page 32, line 27, to page 35 line 17. Newly added claim 36, depends from claim 1, and covers a pyrophosphorylase as the accessory enzyme. Support for this claim is provided, for example, at page 18, line 29, to page 22, line 27 of the specification. Therefore, no new matter has been added.

In the following remarks, Applicants address each of the rejections set forth in the Office Action dated February 26, 2001.

I. Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-27 and 33-35 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in a way to enable one skilled in the art to make and/or use the invention. In addition, claims 1-14, 16-27, and 33-35 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that is not sufficiently described in the specification to convey to one skilled in the art that the inventors possessed the claimed invention at the time the application was filed. Applicants respectfully disagree with these allegations and traverse in part and overcome in part the rejections under this section.

Claims 1-27 and 33-35

With respect to claims 1-27 and 33-35, the Examiner points out that the specification is enabling for a polynucleotide that encodes a fusion protein comprising a specifically identified glycosyltransferase and a specifically identified accessory enzyme. However, the Examiner alleges that the specification is not enabling for a polynucleotide that encodes a fusion protein comprising any glycosyltransferase and any accessory enzyme that catalyzes a step in the formation of a nucleotide sugar that is a saccharide donor for a glycosyltransferase. Specifically, it is alleged that without a specific accessory enzyme, one of skill in the art would not know how to make and use the

glycosyltransferase and vice versa. In addition, the claims allegedly encompass any enzyme involved in any step of biosynthesis of a nucleotide or a sugar. Thus, the Examiner further alleges that undue experimentation would be required to enable the full scope of the claims based upon the instant disclosure.

Applicants respectfully disagree with the Examiner's allegations and assert that Examples 1 and 2 of the specification exemplify how to make a fusion protein comprising an accessory enzyme and a glycosyltransferase. Applicants have demonstrated how to successfully make and use two very different fusion enzymes. In Example 1, Specification pages 38-49, Applicants demonstrate how to make a functional fusion protein by joining a CMP-Neu5Ac synthetase and an α-2,3-sialyltransferase. In Example 2, Applicants demonstrate how to how to make a functional fusion protein by joining UDP-glucose epimerase (end product UDP galactose) and β-1,4-galactosyltransferase. Through these examples, Applicants have demonstrated how to choose appropriate accessory enzymes and glycosyltransferases for fusion, e.i. enzymes that act on the same nucleotide sugar.

Applicants assert that one skilled in the art would know how to identify and select an accessory enzyme that catalyzes the formation of the nucleotide sugar of the donor substrate utilized by the recited glycosyltransferase, without undue experimentation. Likewise, one skilled in the art would know how to identify and select a glycosyltransferase that catalyzes the transfer of a saccharide from a saccharide donor comprising the nucleotide sugar formed by the recited accessory enzyme, without undue experimentation. See, *e.g.*, Guo *et al.*, *Applied Biochem. and Biotech.* (1997) **68**,1-20 (attached). Also see, *e.g.*, the specification, page 12, line 8, to page 23, line 16. It is also well known in the art that glycosyltransferases use a specific and defined group of activated nucleotide sugars that serve as donor substrates. Such activated sugars consist of uridine, guanosine, and cytidine monophosphate or diphosphate derivatives of the sugars in which the nucleoside monophosphate or diphosphate serves as a leaving group. Moreover, the enzymatic activity of both accessory enzymes and glycosyltranferases is

highly specific. Undue experimentation would not be required to make and use the recited glycosyltransferase and accessory enzyme.

To more clearly define the claimed invention, claim 1, and the claims depending therefrom, are amended to recite a fusion protein comprising 1) a catalytic domain of a glycosyltransferase that catalyzes the transfer of a saccharide, from a saccharide donor comprising a nucleotide sugar, to an acceptor molecule; and 2) a catalytic domain of an accessory enzyme that catalyzes the formation of the nucleotide sugar. This amendment is consistent with Applicants' assertions that it is well known in the art that glycosyltransferases have a specific and defined group of activated nucleotide sugars that serve as donor substrates; and that the accessory enzymes and the biosynthetic pathway that catalyze the formation of these particular nucleotide sugars are well-established and highly specific. Thus, one skilled in the art would know how to make and use the recited glycosyltransferase and accessory enzyme, without undue experimentation.

In view of the above arguments and amendments, Applicants respectfully request that the rejection be withdrawn.

Claims 1-14, 16-27, and 33-35

The Examiner points out that claims 1-14, 16-27, and 33-35 are drawn to DNA encoding a glycosyltransferase and an accessory enzyme that catalyzes a step in the formation of a nucleotide sugar. However, the Examiner alleges that the specification provides no DNA sequences or sufficient methods to obtain the claimed DNA. Moreover, it is alleged that there is no correlation that relates the functional characteristics of the DNA to the structure of the DNA.

Applicants respectfully disagree with the Examiner's allegations and argue that the specification is replete with description teaching DNA sequences encoding glycosyltransferases and accessory enzymes, the structure and function of the DNA, and methods for obtaining the DNA (see, *e.g.*, the specification, page 11, line 26, to page 27, line 12). Likewise, the Examples demonstrate a clear correlation between the structure

and function of the DNA, and teach DNA sequences and methods for obtaining the DNA (see, *e.g.*, the specification, page 38, line 12, to page 52, line 6).

For example, the specification describes the structure and function of DNA's encoding glycosyltransferases and accessory enzymes (see, *e.g.*, the specification, page 12, line 8, to page 23, line 16.), and describes how to obtain and clone DNA encoding such glycosyltransferases and accessory enzymes (see, *e.g.*, the specification, page 23, line 17, to page 27, line 12). Moreover, the specification describes DNA sequences encoding specific protein domains of glycosyltransferases (*e.g.*, catalytic domain, cytoplasmic domain, signal-anchor domain, and stem region) and of accessory enzymes, and the function of the encoded protein domains (see, *e.g.*, the specification, page 12, lines 18 to 27; and page 23, line 17, to page 27, line 12).

Further, Example 1 describes the construction and expression of a DNA that encodes a CMP-Neu5Ac synthetase/α2,3-sialyltransferase fusion protein that has both CMP-Neu5Ac synthetase activity and α2,3-sialyltransferase activity (see the specification, page 38, line 12, to page 49, line 8). Similarly, Example 2 describes the construction and expression of a DNA that encodes a UDP-glucose epimerase/β-1,4-galactosyltransferase fusion protein that has both UDP-glucose epimerase and β-1,4-galactosyltransferase activity (see the specification, page 49, line 9, to page 52, line 6). The precise identity or structure of the DNA used to construct the DNA encoding each fusion protein is described in the Examples. Moreover, the results described in Example 1 demonstrate that the CMP-Neu5Ac synthetase/α-2,3-sialyltransferase fusion protein was expressed at high levels and had both of the encoded enzymatic activities (*i.e.*, CMP-Neu5Ac synthetase and α-2,3-sialyltransferase activity); and the results described in Example 2 demonstrate that the UDP glucose epimerase/β-1,4-galactosyltransferase fusion protein was expressed at high levels and had both of the encoded enzymatic activities (*i.e.*, UDP glucose epimerase and β-1,4-galactosyltransferase activity). Therefore, the results described in Examples 1 and 2 demonstrate a clear correlation between the structure and function of the DNA.

In view of the above arguments, Applicants respectfully request that the rejections under § 112, first paragraph, be withdrawn.

Rejections Under §112, Second Paragraph

Claims 4, 9, 33, and 35 are rejected under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Applicants obviate the rejection of claim 4; overcome the rejection of claim 9; and respectfully traverse the rejection of claims 33 and 35, under this section.

Claim 4

With respect to claim 4, it is allegedly unclear how a catalytic domain of a glycosyltransferase would include domains or regions not involved in catalysis, for example, the cytoplasmic domain, signal-anchor domain, and stem region. As stated above, in order to expedite prosecution, claim 4 is canceled without prejudice to subsequent revival.

Claim 9

With respect to claim 9, it is allegedly unclear if the GDP mannose enzymes are grouped as one group of accessory enzymes or as separate members of a Markush Group. In order to more clearly define the claimed invention, claim 9 is amended to insert a semicolon after each recited GDP-mannose and to delete any extraneous punctuation and connectives.

Claims 33 and 35

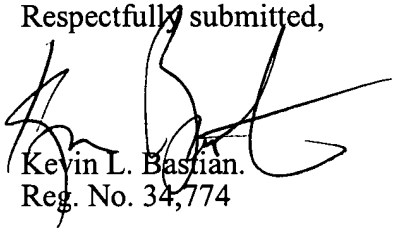
With respect to claims 33 and 35, the claims are allegedly incomplete because they are missing the essential step of isolating the fusion polypeptide. Applicants disagree with this allegation and provide the following arguments. Applicants assert that claims 33 and 35 are directed to a method of producing a fusion polypeptide and that this method is complete once the fusion polypeptide is produced. For example, the fusion polypeptide may be expressed and secreted by the host cell and used in this form without a further step of isolating the expressed and/or secreted polypeptide. Such use is described, for example, at page 33, lines 18-29 of the specification.

In view of the above arguments and amendments, Applicants respectfully request that the rejections under § 112, second paragraph, be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged. If a telephone conference would aid in the prosecution of this case in any way, the Examiner is invited to call the undersigned at 415-576-0200.

Respectfully submitted,



Kevin L. Bastian.
Reg. No. 34,774

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
BLK/KLB
SF 1324203 v1